JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1987, p. 263–267
0095-1137/87/020263-05$02.00/0

Vol. 25, No. 2

# Utilization of Nitrate or Nitrite as Single Nitrogen Source by *Mycobacterium avium*

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Received 28 July 1986/Accepted 15 October 1986

**Twenty L-amino acids and several inorganic compounds were tested individually, as a sole nitrogen source, for ability to support the growth of *Mycobacterium avium* LM1 serovar 1. Of the amino acids tested, only L-glutamine provided nutritional support comparable to that of ammonium chloride at 1 mM. With either 1 mM potassium nitrate or nitrite substituted for ammonium chloride, similar numbers of CFU were produced. *M. avium* cells were grown in potassium nitrate or nitrite concentrations of 0.25, 0.5, 1.0, and 2.0 mM, and the medium was assayed for remaining nitrogen compound at several times during growth. Rates of utilization were of first-order kinetics, with nitrite removed more rapidly than nitrate. The rates were approximately 10 times as rapid at 0.25 mM than at 2 mM for either nitrogen source. Nine clinical isolates that included *M. avium* serovars 1, 4, and 8 and *Mycobacterium scrofulaceum* serovar 43 were tested for rate of utilization of ammonia, nitrate, or nitrite. Ammonia and nitrite were utilized with first-order kinetics by all strains. Nitrate utilization occurred but was not at the same level for all strains. Clinical tests indicate that *M. avium* is negative for nitrate reductase; this is because of the rapid reduction of nitrite produced from nitrate.**

Members of the *Mycobacterium avium* complex include organisms that are widely disseminated in soil (2) and water (4). *M. avium* causes, usually in individuals with some underlying disease, pulmonary disease clinically similar to tuberculosis (15). Rarely, *M. avium* disseminates, as in patients with acquired immune deficiency syndrome, and it may then be isolated from the stool, urine, blood, or bone marrow (9). Most strains that cause disseminated infection are serovars 1, 4, 6, or 8 (5). Although the latter three serovars were previously designated *Mycobacterium intracellulare*, the DNA homology studies of Baess (1) indicated that these organisms should be classified as *M. avium*.

The initial clinical identification of *M. avium* and the serologically related *M. intracellulare* and *Mycobacterium scrofulaceum* is based on the fact that these organisms appear to be metabolically inactive compared with other mycobacterial species. For example, most strains of the three species are negative for nitrate reduction by the routine test (12, 14). Recently, it was found that *M. avium* LM1 serovar 1 could grow on medium that contained a nitrate salt as the only source of nitrogen. The relationship of this metabolic ability to the growth of *M. avium* is reported here.

## MATERIALS AND METHODS

**Organisms.** The mycobacterial strains used are shown in Table 1. *M. avium* LM1 was obtained from a human patient in 1968 and has been the subject of numerous physiologic studies (11). All the other strains were isolated from patients with acquired immune deficiency syndrome and were obtained from the Centers for Disease Control, Atlanta, Ga.

**Media.** Culture medium (CM) was used for batch culture of 1 liter of cells. Basal medium with charcoal-cleaned, dialyzed albumin (BCA) was used for growth of the organisms in volumes up to 100 ml. Ammonia-supplemented (AMM) and nitrogen test (NT) media were designed for use in metabolic studies. In AMM and NT media, palmitate was substituted for oleate, and albumin and $(NH_4)_2SO_4$ were excluded. Individual medium components were prepared at 10- or 100-fold concentrations and diluted in pressure cartridge system (PCS; Barnstead Co., Boston, Mass.) water to form appropriate combinations. The PCS water was obtained by sequential passages of distilled water through a cartridge that contained charcoal for the removal of organic compounds, a cartridge of a mixed-bed ion-exchange resin to remove minerals, and a cartridge to remove particles larger than 0.20 μm. All media and medium components were sterilized by pressure filtration through a filter (0.20-μm pore size) and dispensed into sterile containers.

The basal (B) salts at final concentration contained 13.8 mM $(NH_4)_2SO_4$, 11 mM $KH_2PO_4$, 10.6 mM $Na_2HPO_4$, 1.4 mM sodium citrate, 0.2 mM $MgSO_4$, and 1 ml of a trace element solution per liter. The trace element solution contained ammonium ferric citrate (green) at 40 g/liter, 3.4 mM $CaCl_2$, 3.5 mM $ZnSO_4$, and 4 mM $CuSO_4$. The $(NH_4)_2SO_4$ was not included and $MgSO_4$ was decreased to 0.1 mM in B-1 and B-2 salts; ammonium ferric citrate was excluded from B-2 salts.

CM contained B salts; bovine serum albumin (fraction V powder; Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.), 5 g/liter; oleic acid, 120 μl (dissolved in 10 ml of 0.05 N NaOH) per liter; glycerol, 5 ml/liter; and polyoxyethylenesorbitan monooleate (Tween 80), 1 ml/liter. BCA was the same as CM except that the bovine albumin was charcoal cleaned by the procedure of Chen (3) to remove lipids. AMM25 medium contained B-1 salts; glycerol, 4 ml/liter; palmitic acid, 50 mg (melted in 10 ml of 0.05 N NaOH) per liter; Tween 80, 1 ml/liter; and 25 mM $NH_4Cl$. NT medium was prepared by adding to PCS water the following in the order and at the final concentrations indicated: 60.9 μM $FeCl_3$; B-2 salts; glycerol, 5 ml/liter; Tween 80, 1 ml/liter; and palmitic acid, 25 mg (melted in 10 ml of 0.05 N NaOH) per liter. The individual nitrogen sources were prepared at 100 mM in PCS water and added to NT medium at a final concentration of 1 mM, unless otherwise indicated.

**Culture conditions.** Stock cultures were prepared by inoculating the purified bacteria into 1 liter of CM in a 2,500-ml, low-form culture flask. Such flasks were incubated at 37°C for 14 days and swirled daily. The cells were harvested aseptically by centrifugation and suspended in 10 ml of BCA;

TABLE 1. *M. avium* and *M. scrofulaceum* serovars with culture collection number and strain designation

| Serovar | NMSU[a] no. | Strain designation[b] |
|---|---|---|
| 1 | LM1 | |
| 1 | LM28 | ID 84-550 |
| 1 | LM54 | ID 83-1085 |
| 4 | LM53 | ID 83-640 |
| 4 | LM49 | ID 83-484 |
| 4 | LM51 | ID 83-529 |
| 8 | LM30 | ID 83-1156 |
| 8 | LM56 | ID 83-1146 |
| 8 | LM59 | ID 83-1441 |
| 43 | LM22 | ID 83-1207 |

[a] NMSU, New Mexico State University culture collection.
[b] ID number is the identification number of the Centers for Disease Control, Atlanta, Ga. These clinical isolates from patients with acquired immune deficiency syndrome were provided by Robert C. Good and Mitchell A. Yakrus.

aliquots were distributed into vials, quick frozen, and stored at −80°C.

A cell concentrate of *M. avium* LM1 was removed from −80°C storage, and 0.3 ml was inoculated into 4 liters of AMM25 in an S.M.S. Hi-Density Fermentor (Lab-Line, Melrose Park, Ill.). The bacteria were aerated at 150 rpm for 2 weeks at 37°C in a walk-in incubator. The bacteria were harvested aseptically and stored in 100-ml samples at 4°C. Because of the high concentration of $NH_4Cl$, these cells were mainly coccobacilli less than 1 μm long, and they were used as the inoculum for most of the studies described.

For the comparative studies, the other strains and LM1, stored at −80°C, were thawed, and 200 μl was inoculated into 100 ml of BCA in a sterile 500-ml sidearm flask. These cultures were incubated on a rotary shaker at 150 rpm and 37°C to a density of approximately $1.0 \times 10^8$ CFU/ml. This cell density corresponded to 100 Klett units as determined with a Klett-Summerson colorimeter (Klett Manufacturing Co., New York, N.Y.) with a no. 42 filter.

Cells pregrown in either AMM25 medium or BCA were harvested by centrifugation and washed with B-2 salts that contained 0.1% Tween 80. The cells were inoculated into NT medium, with the nitrogen source as indicated for each study. The cultures were incubated on a rotary shaker at 150 rpm and 37°C, and samples were removed daily for assays.

**Assays.** The turbidity of cell suspensions was determined by $A_{420}$ with a Gilford model 2600 spectrophotometer. The number of CFU per milliliter was determined by diluting each culture sample into S salt solution, which consisted of 15 mM $(NH_4)_2SO_4$, 95 mM $K_2HPO_4$, 44 mM $KH_2PO_4$, 3.4 mM sodium citrate, 0.8 mM $MgSO_4$, and 1 ml of the trace element solution per liter. The dilutions were spotted, 20 μl each, onto 7H10 agar plates. This medium was Middlebrook and Cohn 7H10 agar base (BBL Microbiology Systems, Cockeysville, Md.) supplemented with bovine serum albumin, 5 g/liter; oleic acid, 60 μl (dissolved in 5 ml of 0.05 N NaOH) per liter; glucose, 5 g/liter; and glycerol, 5 ml/liter. The colonies on the plates were counted after 14 days of incubation at 37°C.

Culture samples to be assayed for nitrogen were chilled, and cells were removed by centrifugation either for 15 min at $2,800 \times g$ in a bench-top centrifuge or for 1 min at $15,600 \times g$ in a microcentrifuge. Supernatant fluid was removed, and several different volumes were assayed to obtain determinations in the linear range of the standards. Ammonia nitrogen was determined by the indolphenol blue assay of Keeney and Nelson (8), except that KCl extraction was omitted and the total assay volume was 2.5 ml. Nitrate nitrogen was determined by the brucine method of Jenkins and Medsker (7), except that the assay volumes were 1/10 that described and samples were boiled for 60 rather than 20 min. Nitrite nitrogen was assayed by the procedure of Hanson and Phillips (6), except that the $NaNO_2$ standard solution was prepared with 24.64 mg/100 ml of PCS water and the assay volumes were 1/25 that prescribed.

## RESULTS

**Growth response to single nitrogen source.** The effect of concentration of ammonia on growth and differentiation of *M. avium* LM1 was documented in 1978 (10), and 0.25 mM $NH_4Cl$ was found to be limiting, based on CFU production.

The growth response of *M. avium* LM1 to other nitrogen sources was therefore assessed. The cells, pregrown in AMM25 medium, were washed and inoculated into NT medium that contained a single L-amino acid at 1 mM. Compared with CM or BCA, this medium is deficient as a result of the substitution of a lowered concentration of palmitic acid for oleic acid and the absence of albumin, but it was used because of the nature of the physiologic investigation.

The 20 L-amino acids tested are shown in Table 2, with the results ranked according to the final absorbance reading after 144 h of incubation. The final number of CFU per milliliter for each culture is also shown (Table 2). The cultures initially had an absorbance of 0.0780 and $1.0 \times 10^7$ CFU/ml. There was some increase in absorbance for all cultures. However, compared with the case of the control culture that lacked a nitrogen source, 8 amino acids caused a reduction in absorbance and 11 amino acids provided some growth support. Of

TABLE 2. Response of *M. avium* batch cultured in medium with an amino acid as the single nitrogen source[a]

| Nitrogen source[b] | $A_{420}$ | CFU/ml |
|---|---|---|
| Phenylalanine | 0.1154 | $8.7 \times 10^5$ |
| Threonine | 0.1561 | $6.1 \times 10^6$ |
| Proline | 0.1563 | $4.2 \times 10^6$ |
| Valine | 0.1607 | $4.7 \times 10^6$ |
| Tyrosine | 0.1637 | $3.5 \times 10^6$ |
| Aspartic acid | 0.1719 | $7.3 \times 10^6$ |
| Leucine | 0.1731 | $7.6 \times 10^6$ |
| Serine | 0.1795 | $9.5 \times 10^6$ |
| None | 0.1832 | $5.2 \times 10^6$ |
| Lysine | 0.1896 | $9.0 \times 10^6$ |
| Tryptophan | 0.1963 | $2.9 \times 10^6$ |
| Hydroxyproline | 0.2109 | $8.7 \times 10^6$ |
| Arginine | 0.2102 | $9.5 \times 10^6$ |
| Isoleucine | 0.2117 | $8.0 \times 10^6$ |
| Glutamic acid | 0.2124 | $9.2 \times 10^6$ |
| Histidine | 0.2188 | $1.3 \times 10^7$ |
| Methionine | 0.2388 | $1.4 \times 10^7$ |
| Alanine | 0.2626 | $2.8 \times 10^7$ |
| Asparagine | 0.2816 | $1.0 \times 10^7$ |
| Cysteine | 0.3304 | $9.0 \times 10^6$ |
| Glutamine | 0.7692 | $7.0 \times 10^8$ |
| Ammonium chloride | 1.0146 | $5.7 \times 10^8$ |
| Ammonium nitrate | 0.8273 | $3.6 \times 10^8$ |
| Sodium nitrate | 0.7744 | $4.7 \times 10^8$ |

[a] *M. avium* was incubated in 20 ml of NT medium in a 250-ml flask with each nitrogen source at 1 mM. The results are ranked according to $A_{420}$ of each culture after 144 h of incubation, and the final number of CFU per milliliter is also shown. The initial $A_{420}$ was 0.0780, and there were $1.0 \times 10^7$ CFU/ml at the beginning of the growth experiment.
[b] All amino acids were L-amino acids.

TABLE 3. Growth, determined by CFU and $A_{420}$, of *M. avium* LM1 with nitrate or nitrite as the single nitrogen source[a]

| Nitrogen | Concn (mM) | CFU/ml at: | | | | $A_{420}$ at: | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 25.5 h | 49.7 h | 66.2 h | 97.5 h | 25.5 h | 49.7 h | 66.2 h | 97.5 h |
| None | 0 | $3.5 \times 10^6$ | $2.2 \times 10^6$ | $3.8 \times 10^6$ | $4.3 \times 10^6$ | 0.0599 | 0.1000 | 0.0869 | 0.0366 |
| $KNO_3$ | 0.25 | $1.7 \times 10^7$ | $1.8 \times 10^8$ | $1.4 \times 10^8$ | $1.1 \times 10^8$ | 0.0554 | 0.2730 | 0.3753 | 0.3436 |
| | 0.50 | $1.7 \times 10^7$ | $2.1 \times 10^8$ | $3.2 \times 10^8$ | $4.3 \times 10^8$ | 0.0583 | 0.2531 | 0.4083 | 0.4952 |
| | 1.00 | $1.7 \times 10^7$ | $1.3 \times 10^8$ | $3.3 \times 10^8$ | $3.6 \times 10^8$ | 0.0762 | 0.2372 | 0.3199 | 0.3757 |
| | 2.00 | $9.0 \times 10^6$ | $2.8 \times 10^8$ | $4.9 \times 10^8$ | $5.5 \times 10^8$ | 0.0396 | 0.2307 | 0.3472 | 0.4671 |
| $KNO_2$ | 0.25 | $1.7 \times 10^7$ | $1.5 \times 10^8$ | $1.5 \times 10^8$ | $1.8 \times 10^8$ | 0.0277 | 0.2977 | 0.4021 | 0.4075 |
| | 0.50 | $1.3 \times 10^7$ | $3.0 \times 10^8$ | $3.3 \times 10^8$ | $4.1 \times 10^8$ | 0.0403 | 0.2855 | 0.4533 | 0.5517 |
| | 1.00 | $1.1 \times 10^7$ | $1.6 \times 10^8$ | $3.3 \times 10^8$ | $4.9 \times 10^8$ | 0.0271 | 0.2545 | 0.3927 | 0.4352 |
| | 2.00 | $8.2 \times 10^6$ | $6.7 \times 10^7$ | $2.0 \times 10^8$ | $4.7 \times 10^8$ | 0.0107 | 0.1924 | 0.2925 | 0.3554 |

[a] Samples were removed at the times indicated and plated for CFU. The mean initial CFU was $8.1 \times 10^6$/ml. The mean initial $A_{420}$ of 0.1906 was subtracted from all readings for the results shown here.

the amino acids tested, only L-glutamine (final CFU, $7.0 \times 10^8$/ml) was comparable to $NH_4Cl$ as a nitrogen source; the latter yielded a culture with $5.7 \times 10^8$ CFU/ml (Table 2).

Additionally, $NH_4NO_3$ and $NaNO_3$ were tested as sole nitrogen sources for *M. avium*. CFU production equivalent to that provided by glutamine or $NH_4Cl$ was obtained with either nitrate salt at 1 mM (Table 2). This result for sodium nitrate was unexpected because *M. avium* LM1 was, as are most *M. avium* isolates, negative by the clinical test (12) for nitrate reduction.

**Nitrate and nitrite reduction.** The ability of *M. avium* LM1 to utilize either nitrate or nitrite for growth was examined in greater detail. Cultures were supplemented with potassium nitrate or nitrite at concentrations of 0, 0.25, 0.5, 1.0, and 2 mM (0, 3.5, 7, 14, and 28 μg of nitrogen per ml). Samples were removed periodically for determination of culture turbidity, CFU, and concentration of the nitrogen source.

The CFU determinations (Table 3) had increased at least 10-fold after 2 days (49.7 h) for all nitrogen-supplemented cultures except the culture provided with 2 mM $KNO_2$, which may have been inhibitory initially. There was additional CFU production in all of the nitrogen-supplemented cultures to 97.5 h of incubation, except in those cultures with either nitrogen source at 0.25 mM. There was negligible change in turbidity (Table 3) for the culture incubated in the absence of a nitrogen source, whereas all cultures supplemented with either $KNO_3$ or $KNO_2$ increased in absorbance readings.

Nitrate and nitrite determinations were performed on samples removed at 0, 9, 18, 25.5, 42.3, 49.7, 66.2, and 97.5 h. No nitrite was detected in any of the samples from nitrate-supplemented cultures. With 0.25 or 0.5 mM nitrogen, the supply was exhausted by 25.5 h (Table 4).

The log concentration of nitrogen remaining at each time was plotted versus time, and the result was a straight line, indicating first-order kinetics. Thus, the transport-reductase activities of the growing cells corresponded to concentration and kind of nitrogen in the culture. At each concentration tested, nitrite was utilized more rapidly than nitrate (Table 5). The rate for cells incubated in 0.25 mM was 10-fold that for those in a 2 mM concentration of either nitrogen source (Table 5). That is, the rates for nitrate were −0.0368 and −0.0048 for concentrations of 0.25 and 2 mM and the rates for nitrite were −0.0516 and −0.0067 for 0.25 and 2 mM, respectively (Table 5).

Because these studies were of only a single strain of *M. avium* (LM1), other recent clinical isolates were tested for ability to utilize 2 mM ammonia, nitrate, or nitrite as the single nitrogen source. These included three strains each of serovars 4 and 8, two serovar 1 strains in addition to *M. avium* LM1, and a single strain of *M. scrofulaceum* serovar 43. Samples were removed daily, and determinations were made for culture absorbance and concentration of nitrogen in the medium. The growth of all appeared to be equivalent in terms of absorbance readings and final CFU at 5 days, regardless of the nitrogen supplementation, and none grew in the absence of a nitrogen ion.

Linear kinetics, as evidenced by the correlation coefficient, were obtained for ammonia and nitrite for all representatives of serovars 1, 4, and 8 (Table 6). Nitrate utiliza-

TABLE 4. Nitrate and nitrite determinations during growth of *M. avium* LM1 with either as the single nitrogen source[a]

| Nitrogen | Concn [mM (μg/ml)] | Nitrogen concn (μg/ml) at the following time (h): | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 9 | 18 | 25.5 | 42.3 | 49.7 | 66.2 | 97.5 |
| $KNO_3$ | 2.00 (28) | 24.3 | 23.0 | 19.4 | 18.7 | 13.5 | 13.6 | 11.5 | 8.4 |
| | 1.00 (14) | 11.0 | 9.7 | 9.1 | 7.9 | 4.9 | 4.7 | 3.0 | 0.7 |
| | 0.50 (7) | 5.6 | 4.7 | 2.5 | 1.4 | 0 | 0 | 0 | 0 |
| | 0.25 (3.5) | 2.3 | 2.2 | 0.5 | 0 | 0 | 0 | 0 | 0 |
| $KNO_2$ | 2.00 (28) | 26.4 | 27.0 | 26.0 | 24.4 | 15.7 | 14.5 | 12.0 | 6.5 |
| | 1.00 (14) | 14.5 | 11.9 | 10.8 | 7.9 | 4.8 | 3.5 | 1.3 | 0 |
| | 0.50 (7) | 6.8 | 5.5 | 2.3 | 0.2 | 0.4 | 0 | 0 | 0 |
| | 0.25 (3.5) | 3.1 | 2.8 | 2.3 | 0.1 | 0 | 0 | 0 | 0 |

[a] The cultures characterized in Table 3 were tested for remaining nitrogen as described in Materials and Methods. Samples were removed at the times indicated and assayed. A value of 0 means that no nitrate was detected in 500 μl of medium and no nitrite was detected in 160 μl. The initial theoretical values are shown in parentheses.

TABLE 5. Nitrate and nitrite utilization by *M. avium* LM1[a]

| N concn | | Slope for: | |
|---|---|---|---|
| mM | μg/ml | $KNO_3$ | $KNO_2$ |
| 0.25 | 3.5 | −0.0368 (−0.88) | −0.0516 (−0.79) |
| 0.50 | 7.0 | −0.0242 (−0.97) | −0.0357 (−0.84) |
| 1.00 | 14.0 | −0.0118 (−0.97) | −0.0153 (−0.98) |
| 2.00 | 28.0 | −0.0048 (−0.99) | −0.0067 (−0.98) |

[a] The amount (in micrograms) of nitrate or nitrite nitrogen (N) in the medium at each sample time (Table 4) was used to calculate the nitrate or nitrite removal activity. This activity is given as the slope of the regression line obtained with the $\log_{10}$ micrograms of N per milliliter remaining versus time in hours. The correlation coefficient for each is shown in parentheses.

tion was variable during growth, and linear kinetics for utilization were not achieved for several strains (Table 6). However, the rate of nitrite utilization was more rapid than that of nitrate utilization for each individual culture (Table 6). Thus, any nitrite produced during nitrate reduction was removed and not detectable in the nitrate-supplemented medium.

*M. scrofulaceum* utilized ammonia approximately four times faster than any of the nine *M. avium* strains tested. Only *M. avium* LM1 utilized nitrite as rapidly as *M. scrofulaceum* LM22 (serovar 43) in this experiment (Table 6).

## DISCUSSION

Virtanen (13) reported in 1960 the results of an extensive study on nitrate reduction in mycobacteria. Of 12 strains, designated *M. avium*, 5 were strongly positive, 5 were weakly positive, and 2 were negative. None of the 791 mycobacteria tested by Virtanen reduced nitrite (13). Currently used clinical tests indicate that members of the *M. avium* complex are negative for nitrate reduction; this is a result of a slow nitrate reduction with a subsequent rapid nitrite reduction. Thus, nitrite is absent when tested for colorimetrically and the follow-up test for nitrate is positive because of residual nitrate. Because nitrite reduction by *M. avium* appears to be prevalent, this reaction may prove useful for identification purposes in the clinical laboratory. Direct comparison with other species is necessary to determine whether this activity is unique to *M. avium*.

TABLE 6. Comparison of several clinical isolates for utilization of 2 mM ammonia, nitrate, or nitrite[a]

| Strain | Serovar | Slope for: | | |
|---|---|---|---|---|
| | | Ammonia | Nitrate | Nitrite |
| LM1 | 1 | −0.0038 (−0.99) | −0.0019 (−0.93) | −0.0161 (−0.96) |
| LM28 | 1 | −0.0040 (−0.99) | −0.0010 (−0.87) | −0.0040 (−0.95) |
| LM54 | 1 | −0.0045 (−0.99) | −0.0014 (−0.82) | −0.0029 (−0.95) |
| LM49 | 4 | −0.0045 (−0.99) | −0.0016 (−0.95) | −0.0044 (−0.96) |
| LM51 | 4 | −0.0050 (−0.98) | −0.0010 (−0.82) | −0.0044 (−0.99) |
| LM53 | 4 | −0.0033 (−0.99) | −0.0015 (−0.78) | −0.0023 (−0.97) |
| LM30 | 8 | −0.0038 (−1.00) | −0.0020 (−0.83) | −0.0035 (−1.00) |
| LM56 | 8 | −0.0045 (−0.99) | −0.0014 (−0.82) | −0.0034 (−0.95) |
| LM59 | 8 | −0.0050 (−0.99) | −0.0016 (−0.97) | −0.0041 (−0.99) |
| LM22 | 43 | −0.0199 (−0.98) | −0.0026 (−0.97) | −0.0153 (−0.88) |

[a] Cells grown in BCA were washed in nitrogen-free B-2 salts and then incubated in 20 ml of NT medium in a 250-ml flask with ammonium chloride, potassium nitrate, or potassium nitrite at 2 mM or no nitrogen source. No growth occurred in the latter medium. Samples were removed daily (at 0, 16.4, 43.3, 68.8, 95.9, and 119 h), and the medium was assayed for the appropriate nitrogen ion. The rate of loss is given as the slope of the regression line obtained with the $\log_{10}$ micrograms of nitrate or nitrite nitrogen per milliliter remaining versus time in hours. The correlation coefficient for each is shown in parentheses.

Although *M. avium* does not utilize an amino acid (with the exception of glutamine) as a sole source of nitrogen, it can utilize either nitrate or nitrite salts. This metabolic activity is economical for the organism in that only the amount needed for growth is reduced, as evidenced by the first-order kinetic removal of such a nitrogen source from the medium. The variability of nitrate reduction among different strains may ultimately prove to be useful for distinguishing biotypes. On the other hand, the prevalence of the nitrite reductase activity suggests that these organisms use nitrite not only for assimilation but also as a means of detoxification. Enzymologic investigations are needed to distinguish between these two pathways and to determine the interdependence of the nitrate-nitrite reductase activities in this important group of pathogens.

## ACKNOWLEDGMENTS

This work was supported by Public Health Service research grant AI-21874 from the National Institute of Allergy and Infectious Diseases.

The excellent technical assistance of M. L. Taylor is gratefully acknowledged.

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